Improved production of biopharmaceuticals by site-specific cleavage of fusion proteins expressed in *Escherichia coli*.

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ABSTRACT

The recombinant expression of heterologous proteins in microorganisms, such *Escherichia coli*, is often improved by producing the protein of interest translationally linked to another, often unrelated, protein giving rise to a "fusion protein" construct. For many applications it is desirable or imperative to separate the extraneous material from the protein of interest. An increasingly popular approach to this task is the use of site-specific endoproteases to excise the protein product. A number of commercially available site-specific proteases exist, but many are not capable of generating an authentic N-terminus for the product, display unsatisfactory specificity leading to adventitious cleavage of the product, or they are unsuitable for an industrial process.

Mutants of the serine protease α-Lytic protease have been shown to satisfy many of the criteria for an industrially suitable protease and have been applied to the cleavage of some important fusion proteins used in the production of members of the Insulin-like Growth Factor (IGF) family. Lacking from these examples, however, is any viable proteolytic solution for the liberation of human IGF-I from fusion proteins. This has been primarily attributed to the Proline bearing N-terminal tripeptide sequence of this protein, which is known to be refractory to the activity of many site-specific proteases.

It has been suggested that, in the generation of two combinatorial mutant libraries of α-Lytic protease, the preference for amino acids C-terminal to the cleavage site may have been altered. It is the purpose of this work to first determine if such an alteration has been made in any of the mutants so as to

allow cleavage immediately before the N-terminus of human IGF-I, and then to task the lead mutant(s) to the cleavage of the full-length fusion protein. All members of the two mutant libraries were cultured and their activity confirmed and quantified against a generic β-casein substrate in a high-throughput assay. A second high-throughput technique was then employed to query the mutant proteases for their ability to catalyse proteolysis at the required sequence in a peptide model. Finding that many mutants appeared successful at this task, the findings were verified on a longer peptide model of the cleavage site.

Initially the yields achieved by cleavage of the full-length IGF-I fusion protein by a lead candidate mutant α-Lytic protease were not sufficient to satisfy the requirements of an industrial process, despite alteration of the reaction conditions. However, the insight gained from these reactions could be applied to the redesign of the protein structure around the intended site of cleavage, significantly improving site-specific proteolysis. The IGF-I generated by this cleavage has been shown to be bioequivalent to commercial reference standard to cultured mammalian cells and the yield of this process is approximately 5-fold improved over the existing cleavage system.